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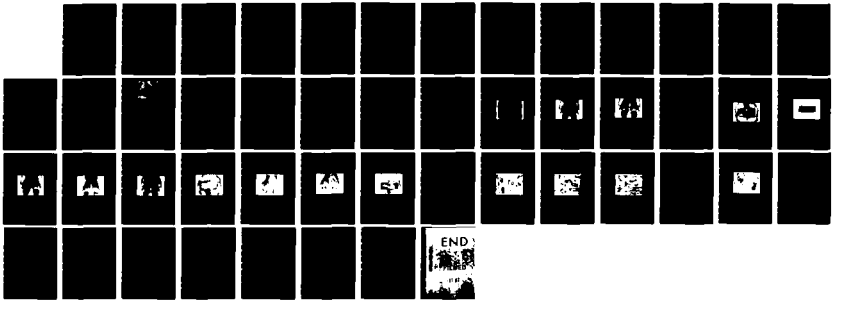

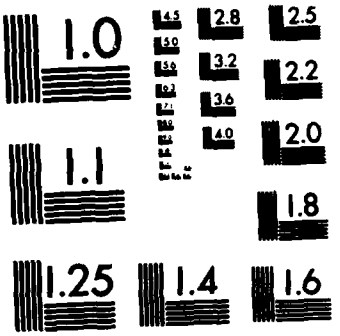

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REPORT NUMBER 6

MANAGEMENT OF HARD TISSUE AVULSIVE WOUNDS AND MANAGEMENT OF OROFACIAL FRACTURES

ANNUAL REPORT

Larry G. McCoy and Craig R. Hassler

August 1980

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DADA17-69-C-9118

BATTELLE
Columbus Laboratories
505 King Avenue
Columbus, Ohio 43201

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Prosthetic Materials	Biodegradable Ceramics	
Implant Materials	Bioresorable Ceramics	

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

This report summarized results of continued studies for further developing and understanding the in vivo behavior of resorbable calcium phosphate for use in the management of hard tissue avulsive wounds and orofacial fractures.

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Specific studies have been devoted to the preparation and comparitive in vivo evaluation of porous tricalcium phosphates having various pore distributions. Secondary studies have included attempts to alter the stoichiometry of the material.

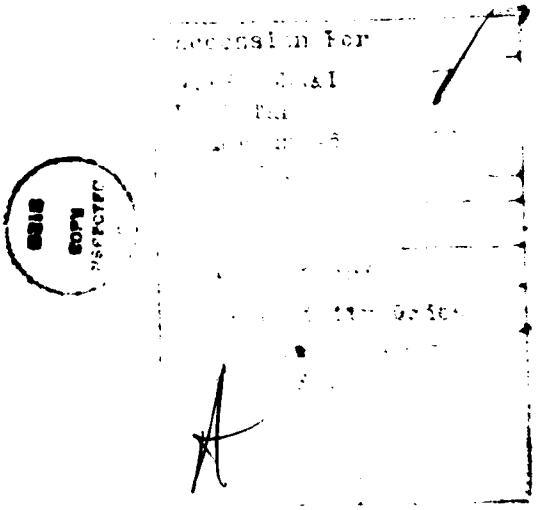
The in vivo studies suggest that the direction of porosity within the biodegradable material is perhaps the most important parameter determining the success of a biodegradable material which facilitates bone ingrowth. It is recommended that a stoichiometric tricalcium phosphate with highly directional pore structure be the object of future research.

ABSTRACT

This report summarizes results of continued studies for further developing and understanding the *in vivo* behavior of resorbable calcium phosphate for use in the management of hard tissue avulsive wounds and orofacial fractures.

Specific studies have been devoted to the preparation and comparitive *in vivo* evaluation of porous tricalcium phosphates having various pore distributions. Secondary studies have included attempts to alter the stoichiometry of the material.

The *in vivo* studies suggest that the direction of porosity within the biodegradable material is perhaps the most important parameter determining the success of a biodegradable material which facilitates bone ingrowth. It is recommended that a stoichiometric tricalcium phosphate with highly directional pore structure be the object of future research.



FOREWORD

This study has been conducted at Battelle's Columbus Laboratories utilizing the talents and resources of the Ceramic Materials Section and the Bioengineering/Health Sciences Section. This is the Sixth Annual Progress Report under Contract No. DADA17-69-C-9118, "Management of Hard Tissue Avulsive Wounds and Management of Orofacial Fractures". The Principal Investigator for this research was Mr. Larry G. McCoy. The physiologist for the animal implant studies was Dr. Craig Hassler.

We would like to acknowledge the valuable assistance of Mr. Roger K. Beal for his excellent work in preparation of the porous implant materials, Mr. Lynn C. Clark for the excellent histologic preparations.

In conducting the research described in this report, the Investigators have adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

SUMMARY

Research studies were continued to further our understanding of the *in vivo* behavior of resorbable calcium phosphate ceramics for use in the management of hard tissue avulsive wounds and orofacial fractures.

Material processing studies were conducted to develop porous tricalcium phosphate materials of different stoichiometry. These two portions of the study were to further understanding of the basic question: Is the optimal material for bone ingrowth and biodegradation going to be produced by alterations in stoichiometry or alterations in pore structure within the material of the given stoichiometry?

Numerous tricalcium phosphate powders were produced having controlled calcium to phosphate ratios. Specifically, three powders were prepared using the standard technique of modifying composition of tribasic calcium phosphate powders. Addition of phosphoric acid was used for the modification. After three powders of various modifications were made at Battelle, the powders were mill-blended and submitted for verification analysis. Materials were then fired and X-ray analyzed by X-ray defraction to determine the crystalline phases that might be present in the finished implants. The results of the study indicated that preparation of a single phase variable composition material does not appear possible using standard methods even though beta phase tricalcium phosphate will be the predominant phase in all materials, secondary phases of monetite or hydroxy apatite were always found depending upon what border of the compositional range the compound fell. Consequently, these three different materials were not developed further. Instead, material of various pore structure confirmation was developed for *in vivo* implant studies.

Three different materials of various pore structures were designed and fabricated into rectangular segments for implanting in the previously used rabbit calivarium model. Animals were implanted and observed for periods of 3, 6, 9, and 12 months, respectively. Each time period, a portion of the animal population was necropsied. Analysis by histology and radiography was performed. In addition, blood and urine calcium and inorganic phosphorous determinations were performed on the animals periodically. The results of the *in vivo* study indicate that modifications within the pore structure are of minimal importance when compared to the direction of pore orientation.

Striking examples of improved bone ingrowth and maintaining stength of the formed bone matrix were found when the pore orientation was in the direction of intended bone growth. That is, from the fresh bone site or fresh cut of bone transversely through the implant material. The analysis suggests very strongly that the optimal material will consist of large diameter porosities through a relatively high strength material, oriented in the direction of desired bone growth.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF CHARTS

TABLE OF CONTENTS
(Continued)

LIST OF FIGURES

MANAGEMENT OF HARD TISSUE AVULSIVE WOUNDS AND MANAGEMENT OF OROFACIAL FRACTURES

by

Larry G. McCoy and Craig R. Hassler

BACKGROUND, PROBLEM AND APPROACH

Historically, various techniques have been employed for the repair or treatment of osseous diseases, defects, or wounds. Autogeneous bone grafting remains the most satisfactory approach but is not without the disadvantages associated with double surgeries and the limitations imposed on the repair of massive osseous defects.

Since April, 1970, Battelle's Columbus Laboratories has been conducting research under contract with the Dental Research Division, U.S. Army Medical Research and Development Command, on the development of resorbable ceramics for potential application in the repair of hard tissue avulsive wounds. The basic materials have been calcium phosphates. These materials were selected because they contain two of the essential elements of the natural bone mineral phase, calcium hydroxyapatite.

In vivo studies were conducted initially at U.S. Army Institute of Dental Research (USAIDR), using the sintered porous materials and slurries prepared at Battelle from tricalcium phosphate $Ca_3(PO_4)_2$ and other calcium orthophosphate powders $CaHPO_4$ and $Ca(H_2PO_4)_2$, to evaluate the potential use of calcium phosphates to both facilitate repair of bone defects and to determine the best material for future exploration[1-3]. The implant studies indicated that calcium phosphates consisting essentially of the mineral phases $Ca(PO_3)_2$, $Ca_3(PO_4)_2$, and $CaHPO_4$ are well tolerated by the tissue, appear to be nontoxic, are resorbable, and permit rapid invasion of new bone.

Of the various porous calcium phosphate materials investigated, tricalcium phosphate, $Ca_3(PO_4)_2$, was selected for continued development and evaluation since it was easy to fabricate and was found to be both biocompatible and resorbable. Emphasis has been directed toward producing low-density porous materials consisting of single-phase tricalcium phosphate[4-7].

Although previous implant studies at USAIDR have demonstrated that porous tricalcium phosphate is biocompatible, resorbable, and promotes or permits rapid ingrowth of new bone, histological evidence indicated persistance of a residual ceramic structure as long as 1 year after implantation. This structure appeared to be composed of an isomorphic distribution of small encapsulated ceramic particles. The presence of this residue would be expected to retard complete remodeling of the bone and the attendant strength development.

As a result of this problem, the primary emphasis of continued studies was directed toward the development of porous materials having improved (increased) resorption rates. This objective may be achieved either by changes in structure or chemistry of the ceramic implant material.

To provide basic resorption rate data on the in vivo behavior of the tricalcium phosphate bioresorbable ceramics, implant studies were initiated in 1975 at BCL using the rabbit calvarium model[8]. Historic samples of tricalcium phosphate were implanted as a control and samples of two new materials were implanted for comparative observation. These new materials were prepared using the improved processing techniques derived in previous materials development studies and represented significant improvements in the structural characteristics of porous tricalcium phosphate. The characterization of the materials involved and the results of the in vivo studies were the subject of the Fifth Annual Report[8].

These results indicated that the improved material exhibited significant increases in resorption rate. In fact, the material resorbed so rapidly that after the ninth month the implant appeared to be granulated and was invaded with connective tissue. This result does not imply lack of biocompatibility but does suggest that such rapid degradation can be deleterious in stress-bearing situations. It is not known whether the enhanced resorptivity resulted from achieving a Ca/P ratio closer to the theoretical for tricalcium phosphate or from the improvements in the structural characteristics of the material.

To discern the effects of structural variations on resorption rate, experimental porous implants were prepared using a single tricalcium phosphate powder but having different pore size distribution. Three materials were prepared for in vivo evaluation.

A second objective of the present program has been to determine the effects of minor variations in chemical composition on the resorption rate of porous tricalcium phosphate materials which have identical pore structures. For this study, it was necessary to prepare new tricalcium phosphate powders having controlled Ca/P ratios. However, to achieve a structurally sound and single phase ceramic, the composition of the new powders must lie within a very narrow β-C_3P (beta tricalcium phosphate) solid solution region.

MATERIALS AND METHODS

Porous Materials Development

Material processing and fabrication studies were continued to develop experimental porous tricalcium phosphate implant materials for in vivo evaluation of the effects of structural variations on resorption rate. The effort has involved the preparation of porous implants of a fixed composition in which the pore size distributions were systematically varied to determine if pore size distributions could be selectively modified to control resorption rate. Three materials were prepared for in vivo evaluation. The characteristics of these materials are summarized in Table 1 and Figure 1.

The fabrication of the Group 1 and 2 materials was completed in the previous program year, the details of which are discussed in the Fifth Annual Report. It was the original intent in the preparation of the Group 3 material to induce a microporosity in the 35-45 micron range and thereby enhance the resorption rate by increasing the permeability and internal surface area of the material. However, histologic evidence from previous implant studies became available during later stages of the material fabrication studies, which indicated that materials having the improved Group 1 type structure had such significantly increased resorption rates that further increases would be hazardous to the mechanical stability of the implant. As a consequence, a new Group 3 material was prepared having a coarser pore structure than the Group 1 material. The intent was to induce rapid bone ingrowth into the larger pores while reducing the resorption by having thicker wall sections between the pores.

TABLE 1. MODIFIED PORE STRUCTURE EXPERIMENTAL IMPLANT MATERIALS

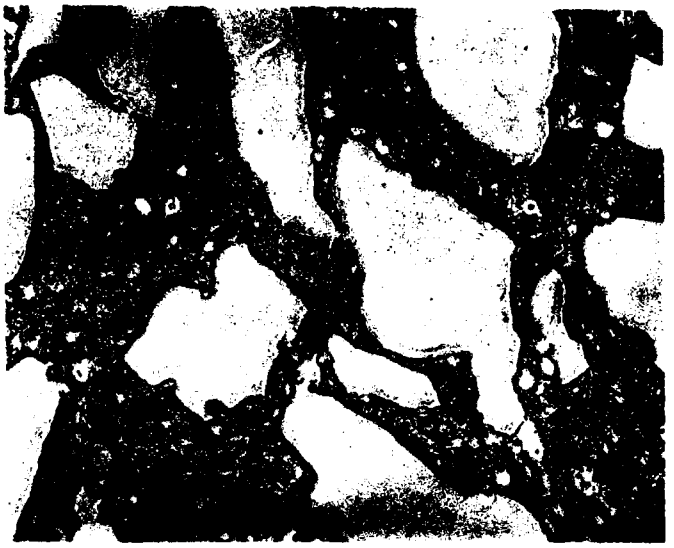
Batch Number[a]	Designation	Naphthalene Pore Size Distribution[b]	Calculated Mean Pore Size (microns)[c]	Sintered Density
E22	Standard	Group 1	260	48.6
E26	Modified-Fine	Group 2	210	47.0
E39	Modified-Coarse	Group 3	290	49.7

(a) All specimens were prepared using Batch D-22 tricalcium phosphate powder and technical grade naphthalene. All specimens were sintered at 2050°F for 4 hours.

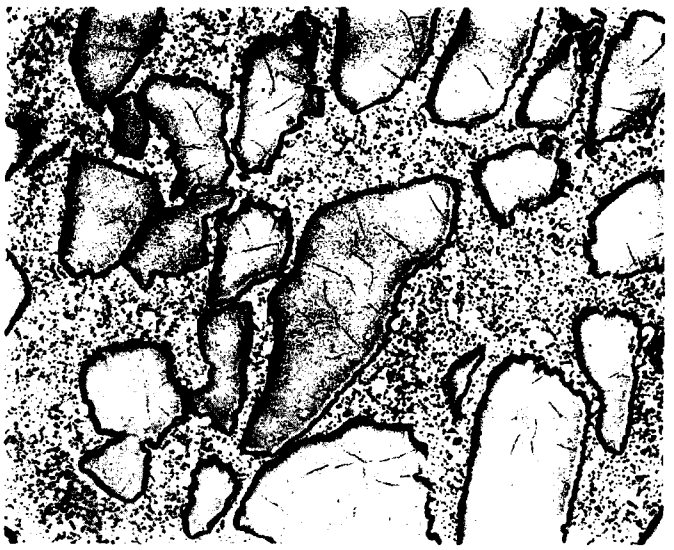
(b) Naphthalene particle size distributions (weight percent):

	Mesh Size -40/+60	-60/+80	-80/+100
	Average Particle Size (microns) 335	213	163
Group 1	76	18	6
Group 2	40	20	40
Group 3	100	--	--

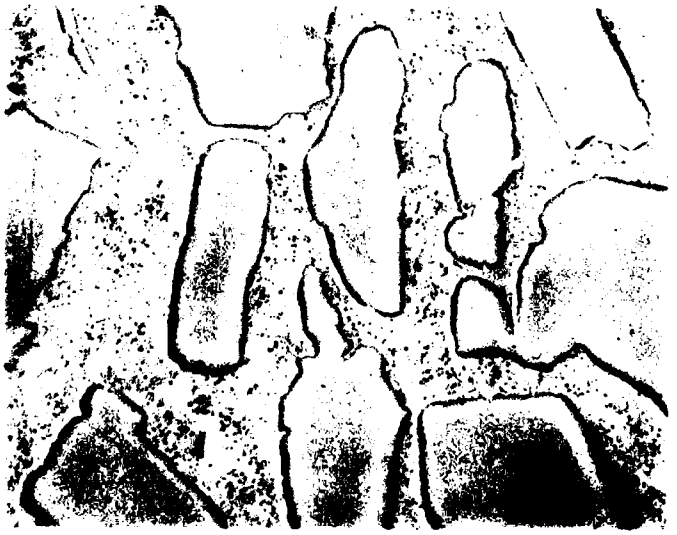
(c) Assuming spherical shape and 15 percent linear shrinkage during sintering.



100X Group 1 Material 8H591



100X Group 2 Material 8H592



100X Group 3 Material 0J888

FIGURE 1. Microstructures of Modified Porosity Tricalcium Phosphate Experimental Implant Materials

The new Group 3 material was prepared by using the coarsest (-40/+60 mesh) fraction of the standard (technical grade) naphthalene distribution. The standard technique of intensive roll blending was used to achieve stable phosphate powder/naphthalene blends. Three blocks of material for sectioning into implants were prepared by the standard hydropressing and sintering procedures used for the Groups 1 and 2 materials. Three implants were cut from the two blocks having the most identical structures (Blocks E39 and E41) and were dry heat sterilized at 600°F for 4 hours.

The preliminary results of the in vivo studies with these materials are discussed in a later section of this report.

Variable Stoichiometry Materials Development

To discern the effect of chemistry, it was sought to develop a tricalcium phosphate having variable stoichiometry. This was based on the observation of the P_2O_5-CaO phase diagram as proposed by Welch and Gutt, which showed a solid solution region at 800°C with a Ca/P ratio of 1.788 to 1.941 for tricalcium phosphate.

For this study, it was necessary to prepare new tricalcium phosphate powders having controlled Ca/P ratios. To achieve a structurally sound and single phase ceramic, the composition of the new powders must lie within the β-C_3P (beta tricalcium phosphate) solid solution region shown in Figure 2. The composition range of the solid solution is quite narrow; the phosphorous content ranges from 20.0 to 20.8 weight percent (45.8 percent as P_2O_5).

Three powders having the compositions designated A, B, and C in Figure 2 were prepared by the standard technique of modifying the composition of tribasic calcium phosphate, $Ca_{10}(OH)_2(PO_4)_6$ by the addition of phosphoric acid. The nature of this method has necessitated reliance on an iterative formulation/analysis/adjustment procedure for producing precise compositions. For an analysis standard, a stoichiometric tricalcium phosphate composition was prepared using a chelometric standard grade of calcium carbonate (100.0 percent $\pm$ 0.05 $CaCO_3$)* dissolved in the appropriate concentration of a certified phosphoric acid (85 percent $\pm$ 0.05 H_3PO_4).

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* Fisher Scientific, Inc., Pittsburgh, Pennsylvania.

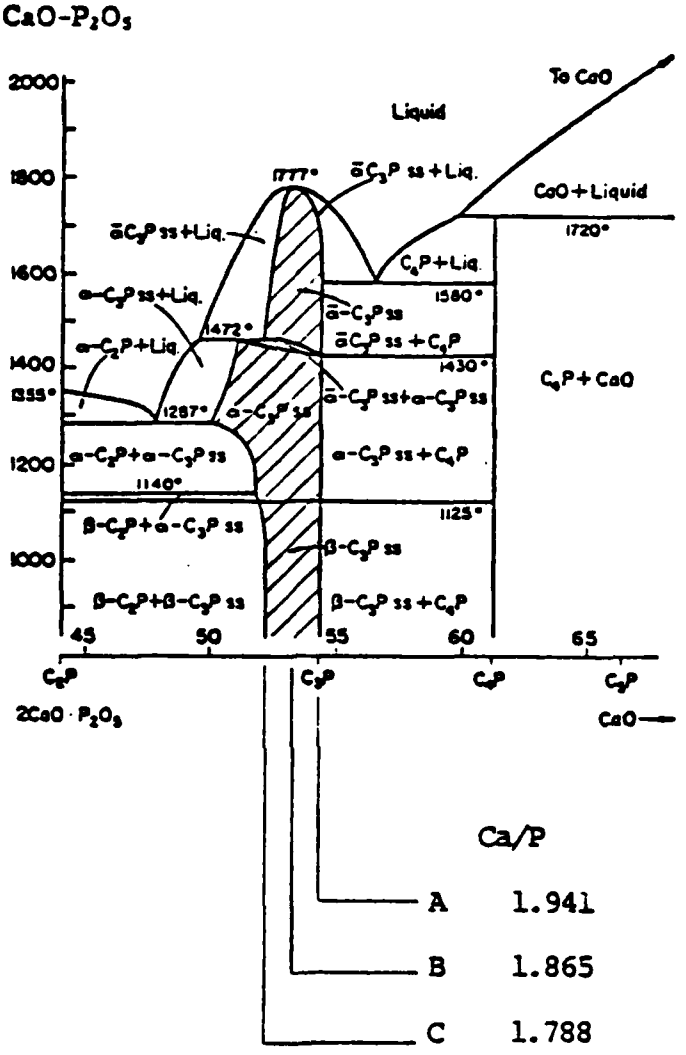

FIGURE 2. System $CaO-2CaO\cdot P_2O_5$ C = CaO, P = P_2O_5

The end member powder compositions A and C were prepared first and were chemically analyzed for total Ca and P contents. Although the absolute values reported for both the Ca and P contents were below the targeted values, when the data were normalized on the basis of the known Ca/P ratios of the analytical standard, the results agreed quite closely with the targeted Ca/P ratio. Powders A and C were then mill blended in equal proportions to prepare the intermediate B composition and samples of all three were submitted for verification analysis. The results of this second analysis were not consistent with the first. As a further check, a third set of samples was analyzed. After normalization on the basis of the known Ca/P ratio of the standard, the results somewhat verified the second analysis (i.e., all compositions were approximately 1 percent calcium rich). However, there was sufficient variation between the results (i.e., approximately 25 percent of the solid solution range) that significant doubt was generated as to the validity of the analysis procedures and/or the ability to produce sufficiently homogeneous powders by the standard procedure.

Although the validity of the above results was uncertain, standard procedures were continued toward the fabrication of porous implant specimens. After the firing, samples of each material were analyzed by x-ray diffraction to determine the crystalline phases that might be present in the finished implant.

Although β-$Ca_3(PO_4)$ was the predominant phase in all the materials, there were secondary phases of either monetite ($CaHPO_4$) or hydroxyapatite $Ca_{10}(OH)_2(PO_4)_6$, depending on what border of the composition range the composition fell. These results suggest that it is not possible to produce a nonstoichiometric tricalcium phosphate with typical low temperature sintering processes. It may be possible to quench melts of CaO and P_2O_5 of the appropriate Ca/P ratio to achieve tricalcium phosphate of variable stoichiometry as is normally the procedure for determining phase diagrams. However, this is not a practical procedure for producing sinterable powders.

Since the preparation of single phase variable composition material does not appear possible, continuation of the chemistry versus resorption characteristics study is obviated. Although it is feasible to conduct implant studies with the present two-phase materials, the presence of these second

phases would only confuse the interpretation of whether any observed effects were due to the tricalcium phosphate or the secondary phases. Consequently, further preparation of these materials was terminated and no implant studies were initiated.

EXPERIMENTAL ANIMAL STUDIES

This portion of the report details the various research procedures which are used in our laboratories to evaluate biodegradable materials. The evaluative procedures include histology, radiography, and blood and urine chemistries. The classical techniques of histology and radiography are the key diagnostic procedures.

Research Protocol

In order to test the biodegradation of large tricalcium phosphate segments, a special experimental model has been devised in this laboratory. We utilize the calvarium of a mature, male New Zealand white rabbit with a minimum weight of 8 pounds. The calvarium has been found to be an excellent implant site for this relatively weak structural biomaterial since stresses upon the calvarium are not extraordinarily high and external stabilization is not required. Consequently, confusing effects which might be due to fixation devices are not seen. Of greater importance is the fact that this implant site provides the researcher with a large, relatively uniform area of material for various simultaneous studies. Additionally, periodic radiography of this flat area is an easy matter.

Standard aseptic surgical technique was used to expose the calvarium of the animal. A rectangular (0.25 inch x 0.75 inch) portion of the calvarium was osteotomized from the animal with no attempt to salvage the periosteum overlying the removed area. To match the curvature of the rabbit calvarium, specifically shaped samples of tricalcium phosphate were fabricated with a thickness of 0.1 inch.

Three different experimental groups of three animals each were followed. These groups were implanted with chemically identical tricalcium

phosphate implants which differed in pore size distribution and orientation of porosity. Four research animals were included in each group. One animal from each group was sacrificed at intervals of 3, 6, 9, and 12 months.

Blood and 24-hour urine samples were taken pre-implant, at each 3-month interval, and at the time of necropsy for all animals and the calcium and phosphorous levels determined. The animals were radiographed at 3-month intervals until the time of necropsy and the excised skulls were radiographed post-necropsy. The histologic analysis consisted of embedding a portion of the excised calvarium and tricalcium phosphate complex in methyl methacrylate and sectioning. The excised sample was stained with basic fuchsin prior to sectioning. Rabbits were stained at 3-month intervals with tetracycline 60 mg/kg, DCAF 20 mg/kg and xylenol orange 90 mg/kg to monitor bone ingrowth. A separate thick section was ground and left unstained for ultraviolet analysis.

RESULTS

Radiographic Examination of Tricalcium Phosphate Biodegradability

Radiographs of the rabbits were taken at 3-month intervals and of the excised skull after necropsy to monitor the biodegradation of the tricalcium phosphate implant. These high resolution radiographs were obtained using fine-grained industrial x-ray film and a Picker Industrial X-Ray Unit. Three animals that are representative of each of the three pore size distribution groups are illustrated.

The radiograph of rabbit C-77 (Figure 3) shows a tricalcium phosphate implant one week after surgery. Note that the implant is readily apparent in the animal's calvarium and distinctly outlined by radioluscence at its borders. This animal is an example of the "group one" pore size distribution. Figure 4 shows the same animal 3 months post-implant. Note that the sample is still observable in the radiograph. However, after 12 months the sample is not visible (Figure 5). This finding is consistent with previous biologic results reported for this project. Namely, due to biodegradation, the radiodensity of the tricalcium phosphate becomes closer to that of bone and is usually not observable after 12 months. However, a different impression of

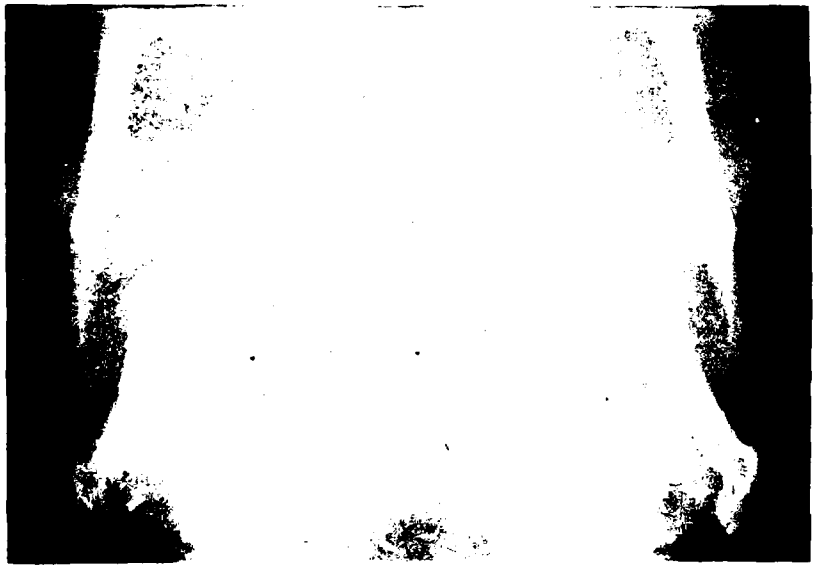

FIGURE 3. Radiograph of Group 1 Tricalcium Phosphate Implant 1 Week Post-Surgery (Rabbit C-77).

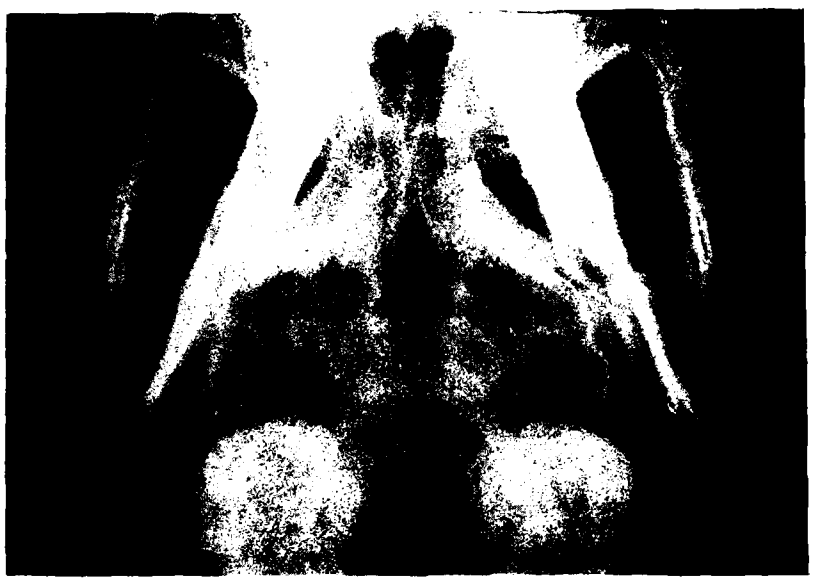

FIGURE 4. Radiograph of a Group 1 Tricalcium Phosphate Implant 3 Months Post-Surgery (Rabbit C-77).

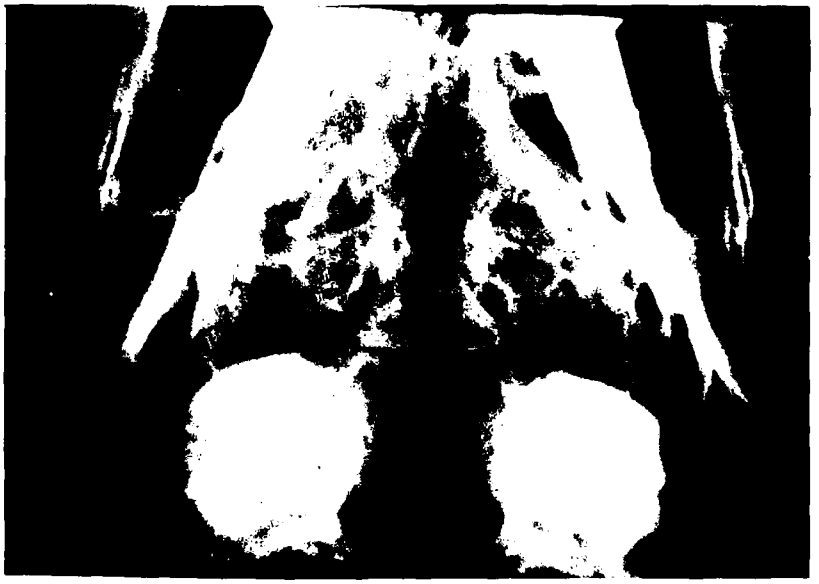

FIGURE 5. Radiograph of Group 1 Tricalcium Phosphate Implant 12 Months Post-Surgery (Rabbit C-77).

the ultimate fate of this material can be obtained if the radiograph of the excised skull is observed. Figure 6 shows the post-necropsy radiograph of rabbit C-77. Much greater resolution is obtained since interfering skin and bone have been removed. It is obvious that biodegradation has taken place. The blotchy appearance of the tricalcium phosphate indicates considerable degradation and a tremendous decrease in density of the implant. In some places holes are left at the borders of the implant where the material has degraded and not been replaced by bone tissue. Other portions of the implant show good confirmation to the surrounding bone. An appreciation for the change in the relative density of the tricalcium phosphate can be made by comparing Figure 6 to Figure 7. Figure 7 is a radiograph of tricalcium phosphate prior to implant. The difference in radiodensity between the pre-implant and 12 months after implantation is obvious.

Figure 8 is a radiograph, one week post-operative, of a rabbit (I-77) that is representative of the "group 2" pore size distribution tricalcium phosphate. It is observed that the implant is similar to rabbit C-77 of group 1. At 3 months (Figure 9), the outline of the implant can be observed only with difficulty and at 12 months (Figure 10), the outline of the implant is no longer discernable. When the excised post-necropsy x-ray of rabbit I-77 is observed (Figure 11), a striking amount of degradation can be observed. Note that large portions of the implant are totally missing and that the remaining tricalcium phosphate is highly granular in nature. It appears as if the implant has totally lost its integrity. Obviously, the desired result of the replacement of the bioimplant with natural bone has not been achieved.

Rabbit J-77 is representative of the "group 3" implant material, and is shown 1 week post-implant in Figure 12. As in the two previous cases, the implant is readily observable with a radiolucent border. In contrast to the two previous material groups, this material is still observable on the radiograph after 12 months (Figure 13). This would indicate a higher density and presumably less biodegradation after 12 months than was observed with the two previous materials. The post-necropsy radiograph of rabbit J-77 (Figure 14) shows considerable degradation has occurred but some of the material is retained. In this particular case, the internal portion of the implant appears to have degraded more than the peripheral portion. This material has apparently maintained its density and integrity to a greater extent than either group 1 or group 2 materials.

FIGURE 6. Radiograph of Excised Skull Post-Necropsy of Rabbit C-77 at 12 Months From Group 1.

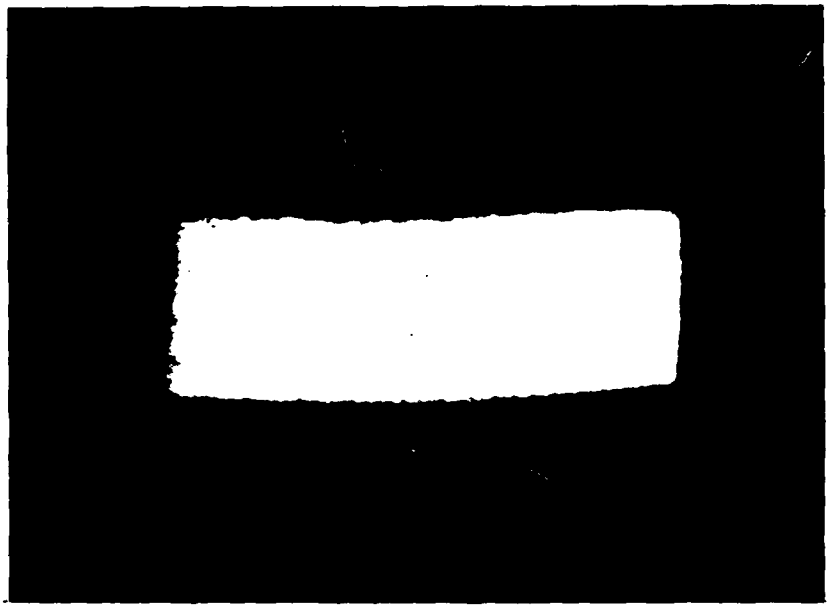

FIGURE 7. Radiograph of Group 1 Tricalcium Phosphate Prior to Implant.

FIGURE 8. Radiograph of Group 2 Tricalcium Phosphate Implant 1 Week Post-Surgery (Rabbit I-77).

FIGURE 9. Radiograph of Group 2 Tricalcium Phosphate Implant 3 Months Post-Surgery (Rabbit I-77).

FIGURE 10. Radiograph of Group 2 Tricalcium Phosphate Implant 12 Months Post-Surgery (Rabbit I-77).

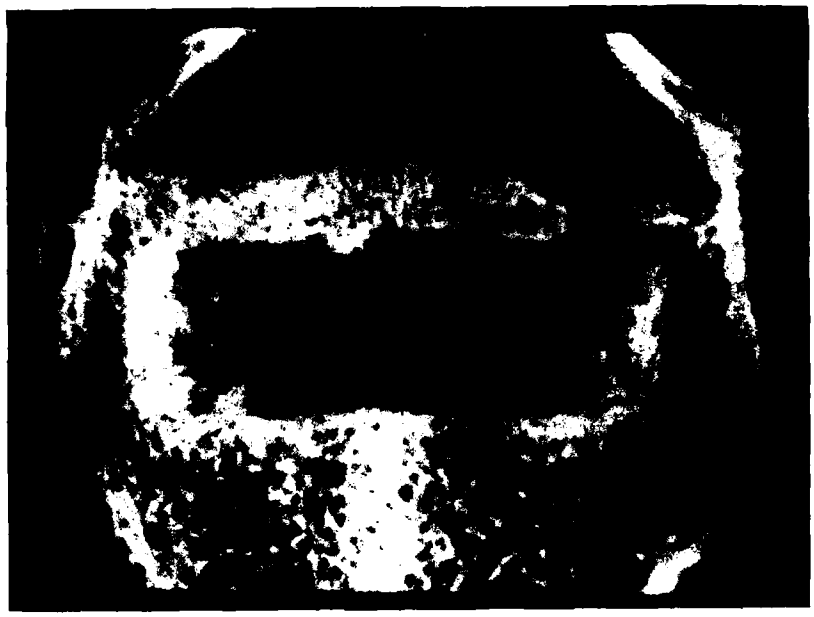

FIGURE 11. Radiograph of Excised Skull Post-Necropsy of Rabbit I-77 at 12 Months From Group 2.

FIGURE 12. Radiograph of Group 3 Tricalcium Phosphate Implant 1 Week Post-Surgery (Rabbit J-77).

FIGURE 13. Radiograph of Group 3 Tricalcium Phosphate Implant 12 Months Post-Surgery (Rabbit J-77).

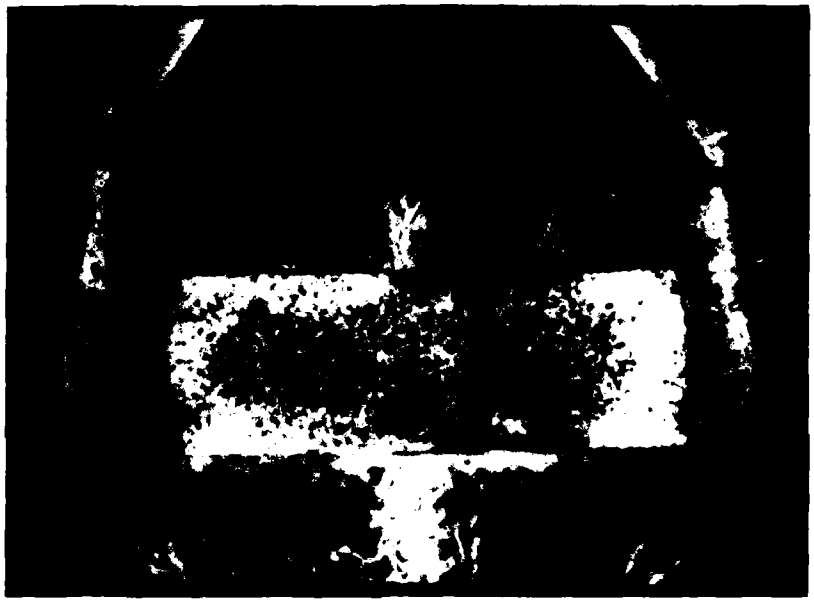

FIGURE 14. Radiograph of Excised Skull Post-Necropsy of Rabbit J-77 at 12 Months From Group 3.

Of the three materials, group 3 appears to have degraded the least and the group 2 material the most. These trends are essentially substantiated by radiography on the other implants in the study. Note that with the group 3 material, the density appears to have been reduced more readily in the middle of the implant instead of at the boundaries, as was seen in the group 1 and group 2 materials.

Histologic Evaluations

To evaluate the rate of ingrowth of biologic material (bone and connective tissue) into the tricalcium phosphate and the subsequent biodegradation of tricalcium phosphate, ground sections of the excised skulls were prepared using a methylmethacrylate imbedding technique. Due to the nature of tricalcium phosphate, sections cannot be prepared without imbedding in a rigid fixation medium such as methyl methacrylate. Slides have been prepared both pre-stained with basic fuchsin and also unstained. The histology illustrated in this report has been stained with basic fuchsin.

Figure 15 is a representative photomicrograph of a "group 1" tricalcium phosphate 3 months post-implant (rabbit A77). This view is near the bone-biomaterial interface. It shows a rich connective tissue within the pores instead of bone. Note that the orientation of the rectangular pores is vertical and is consequently at right angles to the direction from which bone should grow (left to right).

If this ingrowth near the border is compared to an older series of implants with the same chemistry but different pore orientation, one would conclude that the vertical pore orientation is inhibiting ingrowth. This is shown quite clearly by comparing Figure 15 to Figure 16 (rabbit L75) in which the pore orientation is parallel to the direction of ingrowth.

In contrast, Figure 17 shows ingrowth into another "group 1" implant at 6 months (D77) near the bone-ceramic interface. Notice that there is ample bone formulation in the porosity, and that it has a horizontal pore orientation. Consequently two different pore orientations are included in the group 1 samples.

At 9 months, there is good ingrowth of bone in the horizontally oriented porosity and some sequestering at the upper and lower surfaces of the implant. At 12 months a band of bone and porous ceramic is seen through the

FIGURE 15. Photomicrograph of Group 1 Tricalcium Phosphate Implant 3 Months Post-Surgery (Rabbit A-77).

FIGURE 16. Photomicrograph of "Improved Material" Tricalcium Phosphate 3 Months Post-Surgery (Rabbit L-75).

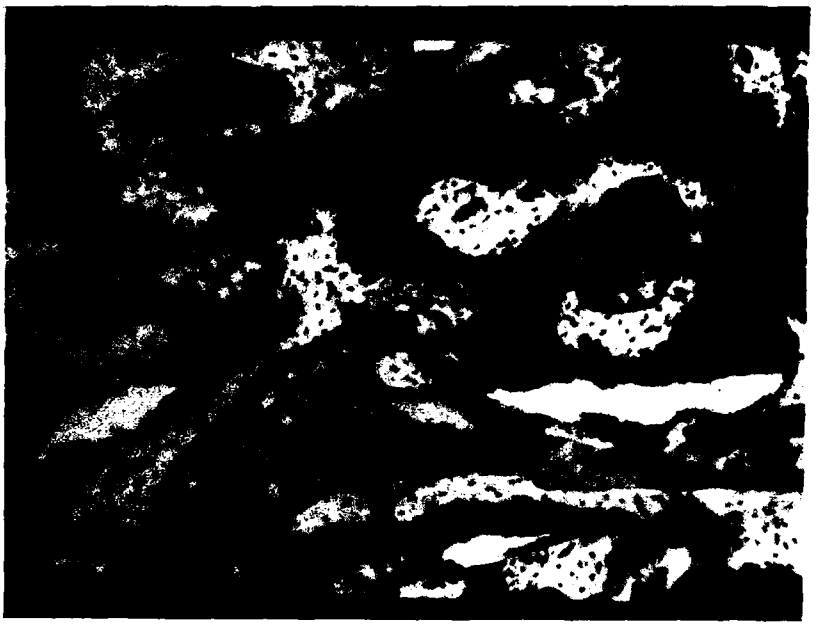

FIGURE 17. Photomicrograph of Group 1 Tricalcium Phosphate 5 Months Post-Surgery (Rabbit D-77).

middle of the implant with some loss of integrity at the upper and lower surfaces. One could speculate that a successful replacement with bone might have occurred with this specimen if there had been a more uniform pore structure and more pore interconnection.

The group "2" material is illustrated in Figure 18 rabbit F77. Pore orientation is vertical. There is little bone tissue after 3 months. At 6 months there is minimal mechanical integrity of the implant remaining. There is some bone tissue seen in a small area which has retained its mechanical stability. At 12 months, the samples in group 2 show no structural integrity and minimal retention of material. Connective tissue invasion is spread throughout the implant which would indicate a "failure" for this material. This "failure" might be ascribed to the too rapid biodegradation of the implant such that there was little matrix remaining for bone ingrowth.

The group "3" material exhibits a definite horizontal orientation of porosity relative to the direction of bone ingrowth. Apparently, interconnection is minimal since little biologic activity is seen except in pores at the specimen edges as evidenced by bone and connective tissue ingrowth. After 6 months, degradation is irregular and the sample is surrounded by bone which has penetrated the peripheral pores of the implant. In some areas degradation is quite advanced, resulting in the sequestering of the remnant material. After 12 months, bone formation is generally seen throughout the sample, which shows only a minimal loss of structural integrity. There is substantial channeling between pores by bone tissue that is attributed to the retention of structural soundness throughout the implant period as well as the high degree of pore interconnectivity. One could speculate that the situation may resolve to a similar outcome as suggested for the group 1 material.

The overall success of the replacement of tricalcium phosphate with bone appears to be strongly related to pore orientation. The slower degradation seen in samples of the group 3 material might provide the structural integrity that is necessary during bone ingrowth. The orientation of the pores in the samples provided a result which was unexpected when the experiment was designed and the biomaterials produced. The pore orientation appears to be an overriding factor which masks any differences which may be caused by pore distribution. The small sample size and differences in pore orientation prevent any firm conclusion as to the importance of pore distribution.

FIGURE 18. Photomicrograph of Group 2 Tricalcium Phosphate Implant 6 Months Post-Surgery (Rabbit F-77).

Quantitative Histologic Analysis

The differences in ingrowth between the various samples were measured by computer digitization of histologic specimens. A summagraphics® digitizer connected to a PDPLSI-11 microcomputer was used. An image of the histologic specimen was projected on the digitizer board. Two distinct areas of the implant in each animal were analyzed: one area was in the center of the implant and the other near the implant-bone interface, a standardized area was measured from each sample analyzed.

The following parameters were calculated:

- percent total porosity (what percent of the observed area is porous),
- percent of pores filled with connective tissue,
- percent of pores filled with bone,
- total percent of pores filled (connective tissue plus bone in pores).

The results for each animal are shown in Table 2. The animals are segregated into groups depending upon the implanted sample group. Insertion time indicates the time duration between surgery and necropsy. Pore orientation indicates the general direction of pores relative to the expected direction of bone growth. For example horizontal orientation would be the preferred orientation for ingrowth from adjacent bone. The percent sequestration refers to areas where the tricalcium phosphate has degraded to a granular consistency and has become engulfed in dense connective tissue. The mechanical stability appears to have been lost in these areas. Samples that are totally sequestered (GE-77 and M-77) could not be analyzed by this technique.

In the group 1 materials, a general increase in percent porosity is seen with time. The trend is more obvious in the middle of the specimen. Trends in connective tissue and bone are difficult to ascertain. However, at 12 months a high percentage of bone was observed in the available pores.

The group 2 material revealed some increase in porosity with time, but an orderly increase in porosity could not be observed at 12 months. Some degree of sequestration was apparent in most samples of this group. The most striking observation is the low percentage of bone seen in the available pores. Since all pores are oriented vertically in this group, there is a strong suggestion of a correlation between pore orientation and bone ingrowth.

RABBIT NUMBER	INSERTION TIME	PORE ORIENTATION	TOTAL SEQ	EDGE OF SAMPLE ANALYSIS (percent) TOTAL POROSITY	CT IN PORES	BONE IN PORES	CT & B IN PORES	CENTER OF SAMPLE ANALYSIS (percent) POROSITY	CT IN PORES	BONE IN PORES	CT & B IN PORES	TOTAL SEQ
GROUP I												
A-77	3 months	vert.		35.85	73.97	18.44	92.41	34.04	61.14	0.00	61.14	
D-77	6 months	hor.		44.88	51.74	45.68	97.42	78.65	40.19	44.73	84.92	
B-77	9 months	hor.	31.97	83.27	59.55	16.41	75.96	63.02	56.09	35.83	91.92	0.00
C-77	12 months	hor.	14.01	64.43	30.28	64.72	96.00	76.43	63.33	24.66	87.99	22.43
GROUP II												
H-77	3 months	vert.		37.69	88.24	0.00	88.24	39.60	63.13	0.00	63.14	
F-77	6 months	vert.		57.07	58.66	36.17	94.83	58.83	84.31	15.53	99.84	
G-77	9 months	mostly vert.	21.61	50.06	39.47	2.75	42.22	69.94	34.23	0.83	35.06	43.15
E-77	12 months	prob. vert. pores	100.00									100.00
I-77	12 months	vert.	75.28	47.06	92.10	8.77	100.00	37.59	32.37	67.71	100.00	70.46
GROUP III												
N-77	3 months	hor.	0.00	17.10	13.66	0.00	13.66	16.05	5.32	0.00	5.32	0.00
K-77	6 months	hor.		58.72	56.69	22.06	89.85	34.35	81.18	0.00	81.18	
M-77	9 months		100.00									100.00
J-77	12 months	hor.	0.00	49.83	16.42	73.64	90.06	65.71	35.95	47.77	83.72	7.87

TABLE 2. Quantitative Histologic Analysis of Tricalcium phosphate. The results were obtained by digitization of the indicated structures. All values are described in percent of a standard area analyzed. Porosity is percent of the observed area. C.T. (connective tissue) is percent of the available pores filled. Bone (B) is described as percent of available pores filled as is the total of connective tissue and bone. Seq is the percent of the total area scanned which is sequested.

The 12 month sample appears to have a high percentage of bone in the pores, but the sample is mostly sequestered and the measurement is thus based on a small area of sample.

The group 3 material exhibits a marked increase in porosity with time, especially in the center of the specimen. Sequestration is low, with the exception of the nine month sample which was totally sequestered. Of special note, is the 12 month sample from this group which exhibited impressive percentages of bone in the available pores. When visually observed, this sample appeared to be the most successful result.

Even though the vertical orientation is clustered in one group the correlation to lack of bone ingrowth is impressive. Since the sample size is small, caution should be used in extrapolating conclusions from this data.

Blood and Chemistry Profiles

Inorganic phosphorous determinations were made using an acid molybdate reaction. Calcium was determined with an orthocresolsphthalein complexone. Reagents were obtained from Dow Diagnostics. Blood was obtained from the animals for serum samples. Twenty-four urine samples were collected from the rabbits by housing them in metabolic cages. Aliquots were removed from a well mixed 24-hour sample for determination. Volume of urine in each 24-hour period was recorded. Multiple determinations were made on all samples. Samples were taken prior to implant, post-implant and then at 3-month intervals until necropsy. Chart 1 shows the averages of blood calcium and the inorganic phosphorous levels on all experimental animals. No significant alteration in either calcium or phosphorous was noted in any of the animals throughout the experimental period. This chart includes data on all of the 77 series animals. This result is similar to that seen in all previous rabbits used in this project. Chart 2 illustrates average urine calcium and inorganic phosphorous levels for these animals. Wide individual fluctuations in calcium and phosphorous levels can be seen throughout. These fluctuations are due in part to the unusual physiology of the rabbit in which the animal produces a concentrated urine which precipitates and cannot always be completely resolubilized. Some individual upward trends can be seen in values post-operatively. This

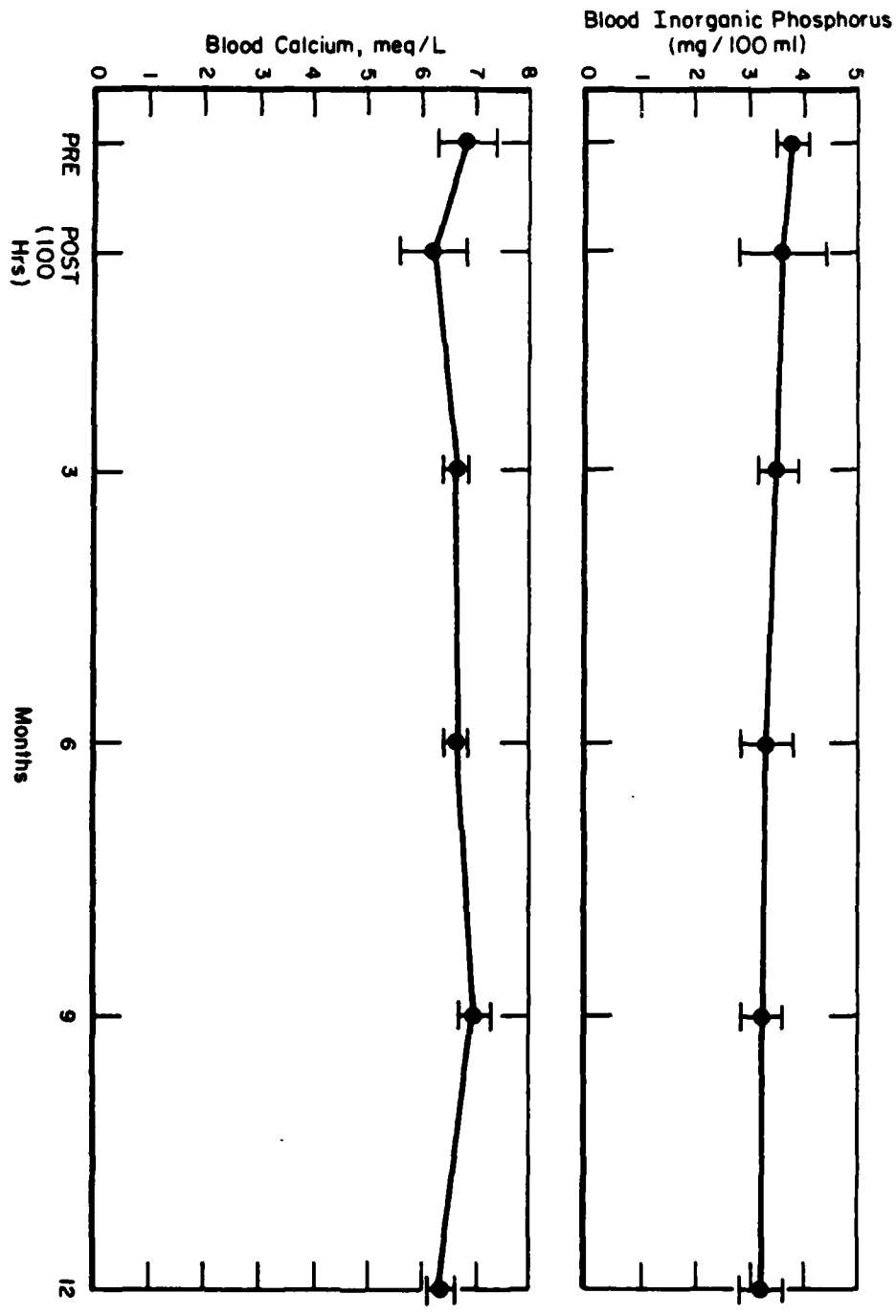

CHART 1. Average Blood Values For Calcium and Inorganic Phosphorous Throughout the Experimental Period.

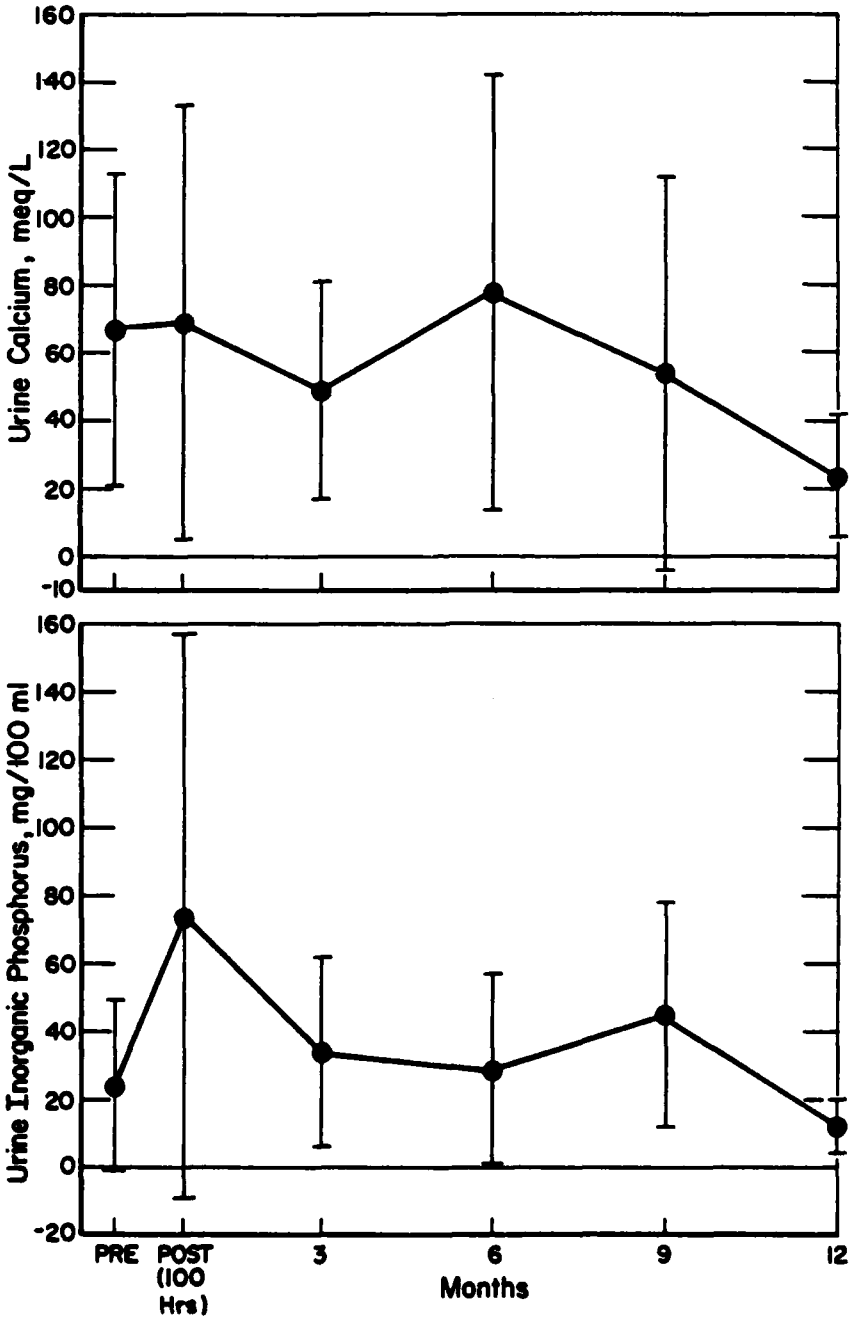

CHART 2. Average Urine Values For Calcium and Inorganic Phosphorous Throughout the Experimental Period.

might indicate if the animal is excreting the excess calcium and/or phosphorous arising from the biodegradation of tricalcium phosphatate. These individual fluctuations are lost in the averaged data due to the extremely wide standard deviations.

Stable blood calcium and phosphorous values indicate the animals can easily handle, within its normal metabolic processes, the excess amounts of calcium and phosphorous being placed in its body pool. There are individual indications of increased excretion, but the unusual physiology of the rabbit limits interpretation.

CONCLUSIONS

The animal studies with tricalcium phosphate indicate that it is a biologically compatible material which can biodegrade. This particular study indicates pore orientation may affect the desired results, i.e., eventual replacement of this tricalcium phosphate bone replacement material with natural bone. Historically, the project to date has shown improvement in the rate of biodegradation as well as an increase in the amount of bone formation into the available porosities. The group 1 and group 3 materials are more "successful" than any of the previous materials produced thus far. However, we have yet to produce a material that provides ideal results. A difficult trade-off is suggested in the data. That is proper selection of material strength, biodegradability and pore structure are necessary so that adequate strength of the newly formed bone is available before the biodegradation of the tricalcium phosphate proceeds too far. The most recent studies indicate that orientation of pore structure is a more important variable than pore size distribution. Assuring interconnection of porosity is another serious problem which can probably be overcome by a redesign of the pore forming technique. The studies point up that a slightly higher density material of the stoichiometric chemistry with directional porosity is probably the desired material.

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